3

Trends in Gene - silencing Research

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1 Introduction

Advance in genome research has provided tools such as DNA microarrays that can be used to analyze the expression and suppression stage in several genes at once. This revealed the fact that not all cells *in vivo* are expressing the same genes in the same manner, but that each cell shows a specific pattern of gene expression. For example, a skin cell and a liver cell each express different genes. This implies the presence of *in vivo* mechanisms for controlling gene expression.

In cancer cells found in cancer-developing tissues or organs, genes that are suppressed in normal cells are expressed while those expressed in normal cells are suppressed, implying the involvement of abnormal gene expression in cancer development. In addition to cancer, abnormal expression or suppression of genes is involved in many lifestyle-related diseases such as diabetes.

Research on mechanisms of gene expression and suppression are showing remarkable growth. Such areas include "gene-silencing research," which involves study of the mechanism of gene suppression without genetic change (mutation) in the genomic DNA itself.

As shown in Table 1, various subjects are studied in gene-silencing research, such as DNA methylation, chromatin modification, structural change in chromosomes and RNA-mediated suppression of gene expression (RNA interference)[1].

Gene suppression mechanism research is active, because the development of techniques for controlling gene expression will lead to the development of new drugs for treating various diseases.

Nucleic acid compounds are chemically synthesized and introduced into cells to suppress gene expression through stoichiometric inhibition of gene transcription or translation. Typical examples of such nucleic acids are single-stranded synthetic DNAs or RNAs and RNA enzymes (ribozymes). Small double-stranded RNAs are attracting attention for their stability and high efficacy in gene suppression.

Unlike conventional synthesized nucleic acid compounds, small double-stranded RNAs suppress gene expression employing a biological mechanism which all organisms retain. This report triggered the advance in gene suppression mechanism research and facilitated the expansion of gene-silencing research.

This report describes the trends in gene-silencing research with the focus being on research on the small double-stranded RNA mechanism, and further discusses the potential of gene-silencing research for drug development. In addition, this paper describes various strategies for further development in this area.

Table1 : Subjects studied in gene silencing research area

Research target	Research subjects
Mechanism of gene suppression without genetic change in the genomic DNA itself	DNA methylation, chromatin modification, structural change in chromosomes, RNA interference (RNAi), siRNA, suppression of gene expression mediated by synthetic nucleic acid compounds

2 The history of gene-silencing research (years 1990-2001)

The present section covers the gene-silencing research (basic research) conducted since the phenomenon was first reported in 1990 until 2001 and introduces breakthrough studies in this area.

2-1 Gene-silencing research originated from studies of plants

The suppression of gene expression called "gene silencing" was first reported in 1990 in studies of plants[2,3].

The phenomenon was discovered almost by coincidence, when the transformation of petunias with transgenes involved in purple pigmentation for artificially producing dark-colored flowers unexpectedly resulted in white flowers. It was considered that the overexpression of transgenes had suppressed the expression of the endogenous pigment genes.

Subsequent research on the mechanism of gene silencing suggests that the phenomenon were not induced by DNA suppression but rather by RNA suppression.

2-2 Gene-silencing research concerning double-stranded RNA started in nematodes

The suppression of gene expression by the introduction of exogenous single-stranded RNAs into cells was first reported in 1985[4], but it was not until 1988 that double-stranded RNAs were found to be directly involved in gene suppression.

The introduction of large (300kbp or larger) double-stranded RNAs into the cells of nematodes suppressed gene expression more selectively and efficiently than in the case of single-stranded RNAs[5,6].

The suppression of gene expression by double-stranded RNAs was named "RNAi (RNA interference)" and has been reported in invertebrates such as hydra, drosophila and plants.

Single-stranded RNAs suppress gene expression by binding to target mRNAs and mechanically inhibiting their translation into proteins. Double-stranded RNAs suppress gene expression using a gene-silencing mechanism that originally existed in vivo but has recently been discover. The mechanism is explained in Section 2-5.

2-3 Discovery of small RNAs as performers of gene silencing

Large double-stranded RNAs introduced into plant or nematode cells are broken down into small double-stranded RNAs of about 22bp within cells. These small double-stranded RNAs induce gene silencing, which was demonstrated in a series of reports published in 1999-2000.

In 2001, an enzyme decomposing the double-stranded RNAs was discovered from drosophila and was named "Dicer"[7], which was also later discovered in mammalian cells[8].

The discovery of this enzyme suggests that gene suppression by double-stranded RNAs is a common phenomenon found in all organisms. The next step was to search for the small double-stranded RNAs that actually function in cells.

2-4 Introduction of double-stranded RNAs also induced gene silencing in mammals

Because the mammalian mechanism for gene silencing is different from those in plants or invertebrates such as nematodes, it is considered that gene suppression mediated by double-stranded RNA is not applicable to mammalian cells. When long double-stranded RNAs of 30 bp or larger are introduced into mammalian cells, the cells mistake it for virus invasion and release interferon, ultimately inducing cell death instead of gene suppression.

Based on the results of gene-silencing studies conducted in nematodes and plants, small double-stranded RNAs of the same size (21 bp) as those decomposed by Dicer, were introduced into mammalian cells instead of the large double-stranded RNAs. This attempt succeeded in inducing gene silencing in various cultured mammalian cells, including human cells, without causing cell death[9].

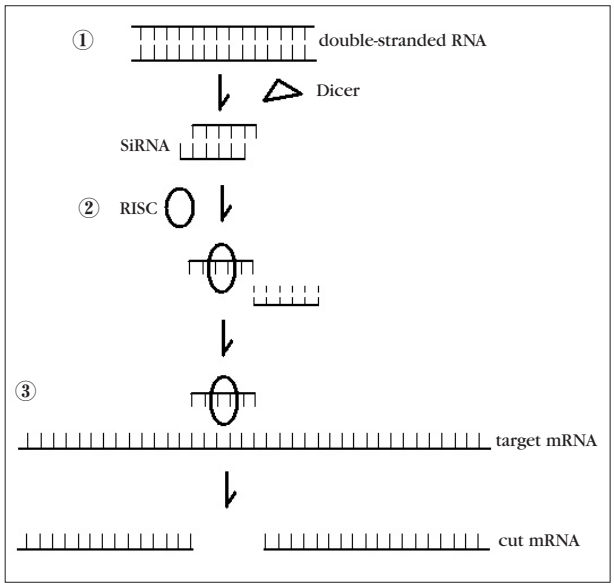
This discovery was a breakthrough in the use of double-stranded RNA in mammals. Small double-stranded RNAs (small interference RNA, siRNA) has rapidly drawn attention as a universal tool for suppressing gene expression.

2-5 *Mechanism of gene silencing mediated by double-stranded RNAs*

Figure 1 illustrates the mechanism of gene silencing mediated by double-stranded RNAs. This is considered a universal mechanism commonly found among all organisms including mammals.

(1) Dicer cuts double-stranded RNA that enter cells into small fragments of about 22 bp to form small interference RNAs (siRNAs).
(2) One of the two strands of the siRNA binds to a RISC (RNA-induced silencing) complex, while the other is decomposed.
(3) The RNA-bound RISC complex binds to a messenger RNA having a sequence complementary to the RNA strand and decomposes the complementary region, resulting in the inhibition of protein synthesis.

Figure 1 : Mechanism of gene silencing mediated by double-stranded RNAs



2-6 *Small RNAs involved in the regulation of in vivo gene expression*

Several small RNAs are present within the cells and are involved in the regulation of gene expression.

These short double-stranded RNAs of 18-25 bp, called miRNAs (micro RNAs), were first reported in 2001[10]. RNAs (pseudodouble-stranded RNA) having hairpin secondary structures are cut by Dicer to form miRNAs. miRNAs consist of mRNA portions corresponding to non-translated regions.

The miRNA suppresses gene expression through a mechanism similar to that of siRNA, but unlike siRNA, which completely binds to the target mRNA sequence, miRNA only partially binds to the target mRNA, resulting in no decomposition of mRNA. Therefore, miRNA is considered to inhibit protein synthesis stoichiometrically. Moreover, since miRNA binds to the non-translated region within the mRNA sequence, it is believed that miRNA acts by binding to a region involved in the control of gene expression.

Hundreds of miRNAs can be found within a nematode or plant cell and are reported to be controlling the timing of development or regulation of stem cells. It is assumed that 200-250 miRNAs are present within human cells[11], and their functions have been actively researched.

Table 2 chronologically lists breakthrough studies in gene-silencing research. As can be seen,

Table 2 : Breakthrough studies in the area of gene silencing research (basic research)

Year	Content	Research target	Highlights
1990	Suppression of flower color gene through gene transfer	Plant	Discovery of modification in phenotypic traits through gene transfer
1998	Gene suppression mediated by double-stranded RNAs (siRNAs)	Nematode	Discovery of a novel gene suppression technique
2001	Discovery of an enzyme (Dicer) decomposing double-stranded RNAs (siRNAs) in vivo	Drosophila	Elucidation of gene suppression mechanism
2001	Gene suppression in mammalian cells by small double-stranded RNAs	Mammal	Discovery of a gene suppression technique for mammalian cells
2001	Discovery of small double-stranded RNAs (miRNAs) considered to be involved in the regulation of gene expression	Mammal	Discovery of a molecule essential to in vivo regulation of gene expression

much of the research was performed in 2001.

3 Overview gene silencing

Based on its own database of research papers called Essential Science Indicators, Thomson Scientific analyzes research papers concerning particular research areas. In December 2003, the company reported the results obtained from their analysis of gene silencing[12].

The present section provides an overview of gene silencing based on the report by Thomson Scientific as well as the results obtained from our own research using the ESI database.

3-1 International comparison of gene-silencing research

Thomson Scientific performed a search on papers published during 1993-2003 using "gene silencing" as the keyword and extracted 1,505 papers written by 4,540 authors representing 898 research institutes from 48 nations and published in 365 academic journals[12] (the papers were searched by keywords by abstracts and authors).

- **The United States is leading the gene-silencing research**

According to Thomson Scientific, the United States was ranked the highest (17,073 citations from 697 papers) for the total number of citations of gene-silencing related papers on a country basis, followed by the United Kingdom (6,374 citations from 168 papers), Germany (3,166 citations from 128 papers), France (2,460 citations from 117 papers) and Scotland (1,716 citations from 47 papers). Japan was ranked 12th, with 649 citations from 111 papers[12].

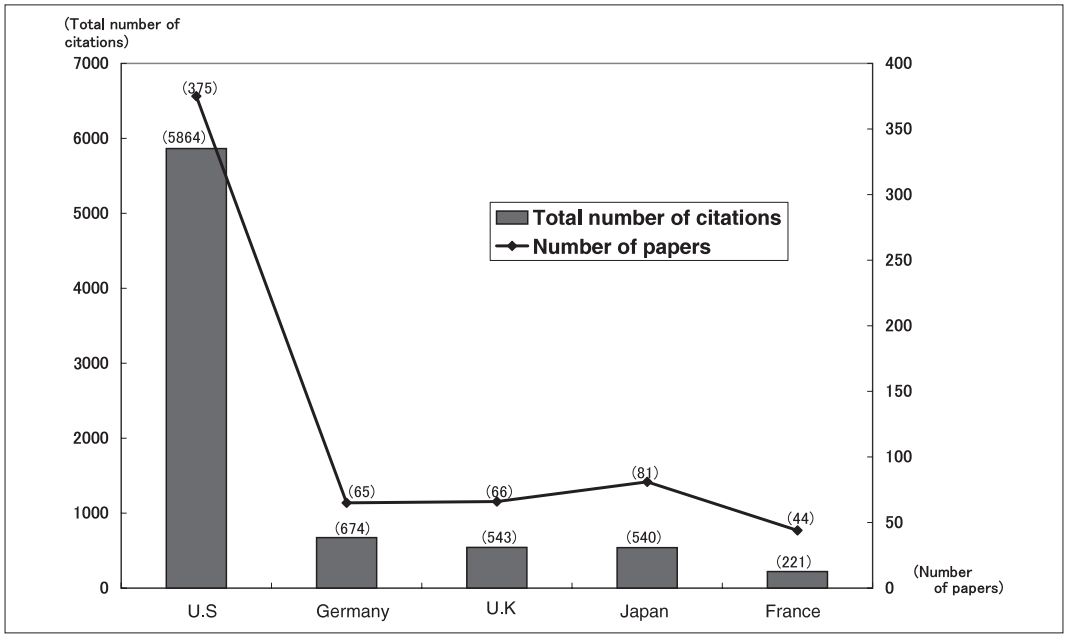
- **The number of citations for Japanese papers are increasing**

In order to compare the numbers of citations on a country basis for the last two years, a similar search was performed using the ESI database.

Figure 2 shows the number of citations of papers published during the last two years (2002-2003) for various countries. As in the analysis performed on years 1993-2003, the U.S. had the largest total number of citations for the last two years.

The total number of citations for Japanese papers increased to a level substantially equivalent to that of Germany or the U.K. Furthermore, about 70% of the papers published during 1993-2003 were published within these two years. This indicates that it has not been long since the research area has become active in Japan.

Figure 2 : Comparison of total numbers of citations of papers in gene silencing research area between countries (2002-2003)



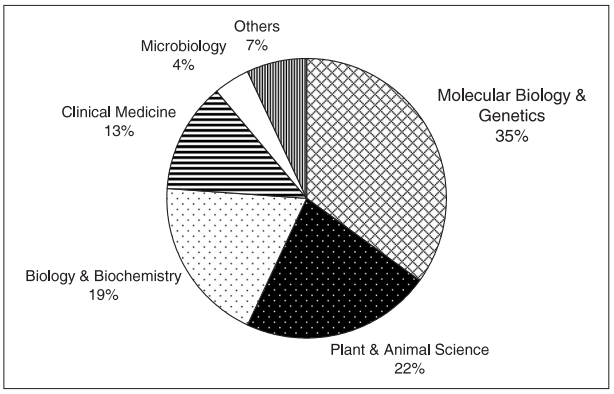
3-2 Characteristics of gene-silencing research

The characteristics of gene silencing were analyzed based on the ESI database.

- **Gene-silencing research is a multidiscipline area**

The papers concerning gene-silencing research are classified by the academic area of the journals in which they are published. As a result, 35% of the papers fall under Molecular Biology & Genetics, while 21%, 19% and 13% fell under Plant & Animal Science, Biology & Biochemistry and Clinical Medicine, respectively[12] (Figure 3).

Figure 3 : Classification of papers in gene silencing research by academic area



The analysis indicates that many academic areas are involved in the gene silencing.

- **The two major subjects dealt with in the ten most-cited papers were DNA methylation and RNAi**

Table 3 lists the ten most-cited papers in gene silencing. The classification of these papers by their research subjects reveals the presence of two major subjects.

One is the mechanism of RNA-mediated gene silencing and the other is the mechanism of gene silencing mediated by DNA or histone methylation found in mammalian cells.

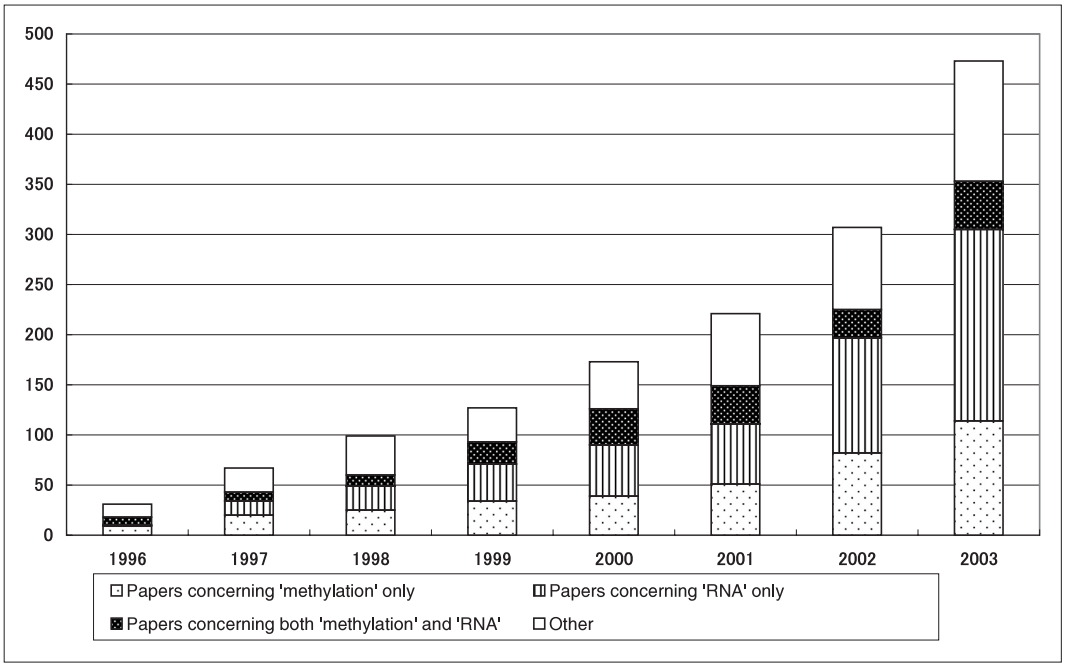
3-3 Shift in gene silencing research

Temporal changes in gene silencing were revealed by analyzing the number of papers using

Table 3 : Ten most-cited papers

	Number of citation	Titles	Subjects	Authors (Nationalities)	Journals (Years of publication)
1	913	Methylation-specific PCR: A novel PCR assay for methylation status of CpG islands STATUS OF CPG.	Methylation	Herma, JG et al. (U.S.)	PNANS (1996)
2	627	Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells.	RNAi	Elbashir, SM (Germany)	NATURE (2001)
3	584	Methylated DNA and MECP2 recruit histone deacetylase to repress transcription.	Methylation	Jones, PL (U.S, Belgium, Italy)	NATURE GENETICS (1998)
4	522	Cancer epigenetics comes of age	Epigenetic	Jones, PA (U.S.)	NATURE GENETICS (1999)
5	411	Methylation of the 5'-CpG island of the P16/CDKN2 tumor-suppressor gene in normal and transformed human tissues correlates with gene silencing.	Methylation	Gonzalezzulueta, M (U.S.)	CANCER RESEARCH (1995)
6	345	A species of small antisense RNA in posttranscriptional gene silencing in plants.	Antisense RNA	Hamilton, AJ (U.K.)	SCIENCE (1999)
7	339	Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain.	Methylation	Bannister, AJ (U.K., Scotland)	NATURE (2001)
8	308	Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins.	Methylation	Lachner, M (Austria)	NATURE (2001)
9	293	An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells.	RNAi	Hammond, SM (U.S., U.K.)	NATURE (2000)
10	264	RNA interference is mediated by 21-and 22-nucleotide RNAs.	RNAi	Elbashir, SM (Germany)	GENES & DEVELOPMENT (2001)

Figure 4 : Shift in the number of papers and research subjects in the gene silencing research area (1996-2003)



the ESI database.

The number of papers published in each year was investigated using "gene silencing" as the search keyword. Only 31 papers were published in 1996, but the number of papers has continuously increased since then and drastically increased since 2001 (Figure 4).

In addition, as mentioned in Section 3-2, since "RNA" and "methylation" were the major subjects researched in the most-cited papers in the gene-silencing research, these keywords were each combined with "gene silencing" to examine the temporal shift in research subjects within the area (Figure 4).

The result demonstrates that the mainstream has shifted from methylation to RNA research; the former was dominant until 1997 while the latter became dominant after 2002. This reflects the shift of researchers' interest into small double-stranded RNAs after the mechanism for RNA-mediated gene silencing was fundamentally understood by 2001.

Furthermore, many reports suggesting the mutual involvement of "RNA" and "methylation" in the gene-silencing mechanism were successively published in 2004[13-15]. Therefore, the two subjects that were dealt with separately are likely to be integrated into a single research area.

4 Development of applied research on gene silencing toward disease therapy (2002-2004)

Experiments using mammalian cells or mice have been conducted in expectation of the development of new drugs and therapeutic or prevention methods using the small interference RNAs (siRNAs)[16]. The target candidates are diseases that are potentially cured by suppressing gene expression, such as virus diseases (e.g. HIV/AIDS, influenza, SARS, virus hepatitis), neurologic diseases (e.g. Parkinson's disease, Alzheimer disease), cancer and autoimmune diseases (e.g. rheumatism).

Table 4 lists the breakthrough studies that contributed to the development of such applied studies.

4-1 Research on disease therapy by gene silencing

The following cases are examples of applied research on gene silencing performed using animal models for human diseases.

- **Inhibition of HIV virus infection**

In rapidly dividing cells, the absolute number

Table 4 : Breakthrough studies in the area of gene silencing research (applied research)

Year	Content	Research target	Highlights
2002	Gene suppression by injection of small double-stranded RNAs into mice through tail vein [17]	Mice	Successful in vivo delivery through an ordinary means
2003	Prevention of hepatitis using small double-stranded RNAs in hepatitis model mice [18]	Mice	Establishment of the principle for therapies mediated by small double-stranded RNAs
2004	Discovery of a novel molecule from a known signaling pathway through an RNAi-based screening system [19]	Human	Establishment of a large-scale screening system enabling search for novel targets for drug

of siRNAs per cell decreases as the cells divide and propagate, so the effect of siRNA-mediated gene silencing only lasts for about 5 days. This was considered a drawback of siRNAs when using them as drugs, but research conducted in 2003 provides a solution to this[20].

HIV viruses invade the macrophages by binding to CCR5 receptors present at the macrophage surface, so the inhibition of their binding to the receptors should prevent their infection. Moreover, macrophages do not undergo cell division, so siRNA-mediated gene suppression was expected to last over the long term.

siRNAs that suppress the expression of the macrophage CCR5 receptor genes and siRNAs that suppress the expression of HIV virus genes were produced and used in combination to test their effectiveness in preventing HIV virus infection.

The preliminary introduction of the siRNAs suppressing the CCR5 receptor gene expression into macrophages successfully prevented HIV-infection of the macrophages. Moreover, siRNAs suppressing the expression of HIV virus genes proved effective for preventing the replication of viruses that have already infected the macrophages. These results demonstrate the potential of siRNAs, not only as preventive drugs but also as therapeutic drugs.

- **Prevention of aggravation in liver diseases**

Many liver diseases are accompanied by apoptosis (cell death) in liver cells mediated by Fas protein. The death of liver cells deteriorates liver function, resulting in symptoms such as liver cirrhosis. Therefore, the prevention of cell death in liver cells by suppressing the expression of Fas protein should prevent the progression to severe status such as liver cirrhosis.

In 2003, siRNA-mediated gene silencing was reported in autoimmune hepatitis model mice. Development of hepatitis in the hepatitis model mice was successfully prevented by injecting siRNAs that contain sequences corresponding to the Fas gene via their tail veins[18].

4-2 Drug development research by gene silencing

Instead of being used as drugs, the small interference RNAs are used as tools for searching in vivo targets (proteins) for developing new drugs.

- **Research on cancer drug development**

In 2003, de-ubiquitinating enzymes in cancer-related pathways were identified using RNAi. The result demonstrates that the inhibition of familial cylindromatosis tumor suppressor gene (CYLD), having no known function, enhances the activation of the transcription factor NF-κB and imparts resistance to apoptosis (cell death)[21].

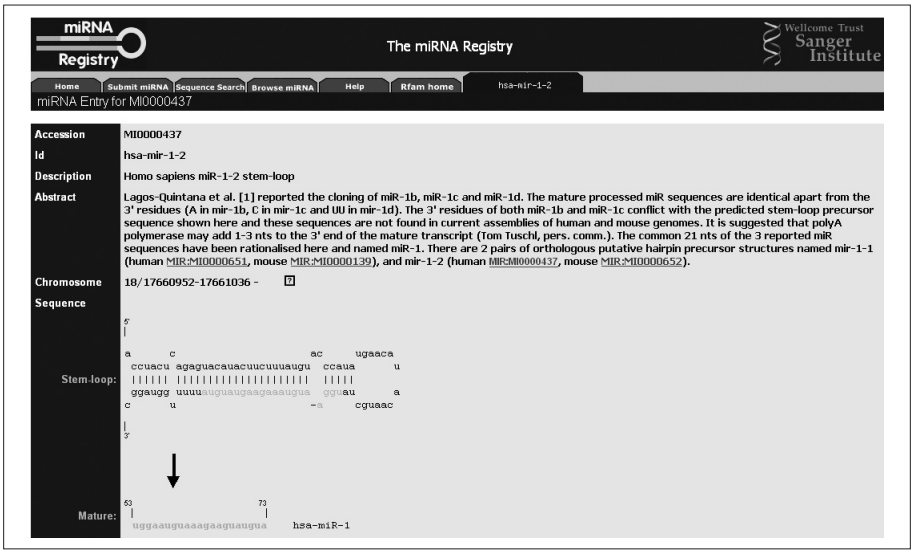
This implies that the loss of CYLD inhibits cell death and therefore inhibits the removal of abnormal cells, which leads to the accumulation of genetic mutations and develops cancer. Although the relationship between the loss of CYLD and oncogenesis has been recognized, the mechanism of CYLD-mediated oncogenesis is not understood.

Based on this research result, NF- κB inhibitors are researched as potential drugs for treating familial cylindromatosis.

4-3 Infrastructure development for promoting gene-silencing research

Databases concerning the small interference RNAs are established mainly in the U.S. and European countries to provide information such as RNAi sequence data.

Figure 5 : miRNA database



Source: The miRNA Registry website

• Databases of small interference RNAs (miRNA)

The British Sanger Institute (funded by Wellcome Trust) has established a database of small interference RNAs[22,23]. To date, 899 miRNAs have been registered, and data including 78 drosophila, 166 nematode (two species) and 191 human miRNAs can be freely accessed (Figure 5).

4-4 Trends concerning drug development

Gene-silencing research has passed the applied research phase and now entered the phase of clinical studies and drug development.

• Corporate-driven technical cooperation has been established for drug development

Many universities and companies forged technical partnership around 2003. Examples of such partnership are presented in the following section.

A U.S. company Sirna Therapeutics, Inc. entered into a license agreement with the University of Massachusetts Medical School concerning small interference RNA (siRNA) technology.

Merck & Co. Inc. and Alnylam Holding Co. collaborated to develop RNA-based technology and therapeutics. Alnylam Holding Co. is a merged company of Alnylam Pharmaceuticals Inc. and a German company Ribopharma AG. Ribopharma AG, who already had many European patents concerning RNAi, aimed at acquiring RNAi-related U.S. patents by merging with Alnylam Pharmaceuticals Inc.

Furthermore, Alnylam Pharmaceuticals Inc., the pharmaceutical division of the Alnylam Holding Co., formed a research collaboration with Mayo Clinic, the leading comprehensive medical institute in the U.S. functioning both as a general hospital and as a research and education institution, to develop therapeutic drugs for Parkinson's disease using RNAi-based technology.

To date, no clinical study (clinical trial) such as human administration of siRNA drugs has been reported, but it is expected to begin in the near future (by the end of 2004 or 2005).

5 Unresolved research issues concerning gene-silencing research

The present section discusses the unresolved research issues for the medical application of gene silencing and suggests research required for their resolution.

5-1 Unresolved research issues in basic research

In gene-silencing research, many breakthrough studies at the basic research level were reported within a short period. This gives the impression that sufficient basic research has already been accomplished, but there are many aspects that are

not fully understood.

- **Elucidation of RNA interference (RNAi) mechanism**

Some parts of "the mechanism of gene silencing mediated by double-stranded RNAs" described in Figure 1, such as the detailed structure of the RISC complex or its function, remain unclear. Further research is required to elucidate the details of the mechanism.

- **The roles of small interference RNAs (miRNAs) in vivo**

Recent studies reveal that a mammalian cell contains about 250 miRNAs, which are assumed to be controlling the expression of various genes in vivo.

These small interference RNAs (miRNAs) are assumed to be controlling the expression of genes in various steps of development and differentiation or in various organs.

Some miRNAs act as switches for turning on and off gene expression. While, some proteins have functions of "suppressing" other proteins *in vivo*. When the expression of genes for such proteins is suppressed by the miRNAs, the other proteins are freed from the "suppression" and regain their "expression."

The elucidation of the functions of all 250 miRNAs in a mammalian cell should lead to the elucidation of the *in vivo* mechanism of gene regulation and the network among genes. However, most miRNAs have not been functionally identified.

- **Relationship between suppression of gene expression and RNAi in chromosomes**

A normal chromosome has regions where the expression of particular genes is suppressed by methylation. An unstable gene suppression status prevents normal development or differentiation or induces diseases such as cancer or congenital abnormality. Recent reports suggest that such chromosomal gene suppression involves an RNAi-mediated mechanism, but the actual mechanism is unknown.

The control of gene suppression status in chromosomes should provide novel findings in development and differentiation studies and ultimately bring a breakthrough in the development of new drugs for treating diseases. It is therefore important to elucidate this mechanism.

5-2 Unresolved research issues concerning the applied research toward medical application

Since RNAi drugs have not been developed, it is difficult to estimate the size of the market concerning drug development using RNAi. RNAi is currently applied mainly to experimental techniques or reagents for research, and its market size is estimated to be $300 million. Such techniques should be involved in the development of therapeutic drugs or techniques, so the current value of the drug development market using RNAi is estimated to be $500 million. By 2010, the market is expected to increase to $1 billion[24].

Meanwhile, many research issues must be resolved before the practical application of RNAi to drugs.

- **The issue of in vivo drug delivery system (DDS)**

The drug delivery system (DDS) is an important issue for using siRNA as drugs. The siRNAs administered through ordinary means must be securely delivered to the target organs, stably exist *in vivo* until therapeutic effects are achieved and exert the effects in a sustained manner.

Some of the possible means for delivering the siRNAs in vivo are (1) the use of synthetic polymers such as liposomes as carriers or (2) the use of safe virus vectors as carriers. Such means have been discussed in gene therapy research, but sufficiently safe and efficient techniques have not been achieved.

Nanotechnology-based techniques such as the use of nanoparticles as carriers are promising DDS techniques that may be developed in the future.

- **The issue of side effects**

Highly selective drugs are thought to posses few side effects. However, even among the drugs recently developed as molecular-targeting drugs with few side effects, some drugs have

been reported to exert severe effects in certain patients. In the case of gene-targeted drugs, certain combinations of the drug and the genetic status of the patients may produce unexpected side effects.

Moreover, even though the human genome has been decoded, the overall network among genes and their functions in vivo is fully understood. This means that there is a risk that the suppression of a certain gene may induce unexpected suppression or expression of other genes.

In addition to mRNAs, cells contain small RNAs present within the nuclei involved in the biosynthesis of ribosomal RNAs. The RNAi drugs introduced into the cells may be transferred into the nuclei and bind to these small nuclear RNAs[25].

5-3 Research promoting medical application of gene silencing

Drugs based on gene silencing directly or indirectly acts against the genes. Therefore, their safe, effective use requires sufficient understanding of in vivo functions of the major genes, the regulatory mechanism of their expression and the network among genes.

The following research is suggested for understanding such subjects.

(1) Basic research using model organisms

The function of vital genes are common among all living organisms, so detailed studies using model organisms should provide results applicable to humans.

Extensive research has been conducted on experimental organisms such as nematodes and drosophila, but even in such organisms, little is understood regarding the vital functions responsible for life itself, including the genes.

(2) Basic research applying problem-solving approaches

In order to promote the medical application of gene-silencing research, it is important to solve research problems that are created in applied or clinical studies. For this purpose, it is effective to conduct basic research taking problem identification and problem-solving approach.

(3) Applied and clinical studies according to needs

The medical application of gene silencing requires information of the patient types, their needs and the suitability of the gene silencing-based treatment for individual cases.

Applied and clinical studies must reflect the needs from the actual clinical environment, which requires the establishment of a system for efficiently providing the researchers with medical information.

6 Conclusions

Finally, this section characterizes the gene silencing and suggests measures to be taken for further promotion of the area.

6-1 Characteristics of this area

The following three points characterize the research area.

- **The fusion of several research areas has promoted gene-silencing research**

Gene silencing has grown rapidly over the past 4-5 years. The area has expanded through the fusion of several basic research areas such as DNA methylation and RNA-mediated gene suppression. The research targets were first limited to plants but were soon expanded to yeast and nematodes. Now the research subjects have shifted from mice and other mammals to human disease therapy.

- **Transition of the research phase is quick**

In most cases of biomedical research, the research phase has shifted from basic research to applied research and ultimately to clinical research. This shift of the research phase has been very rapid in gene silencing (Table 5).

- **The U.S. leads the area, but the number of citations of papers by Japanese authors is increasing**

Analysis of research papers concludes that the U.S. plays a leading role in this research area. However, the number of citations of papers by Japanese authors is increasing, which implies the expansion of scale of domestic gene-silencing research.

Table 5 : Research phases in gene silencing research

Research phase	Year	Period	Research subject
The first report	1990	(8 years)	The first report on gene silencing
Basic research	1998 – 2001	4 years	Elucidation of in vivo mechanism of gene silencing
Applied research	2002 – 2004	3 years	Research using animal disease models toward medical application
Clinical research	2005 – ?	?	Research concerning clinical trials performed on patients

Among the papers published in the top journals having impact factors of 10 or higher in 2002-2003, papers involving Japanese researchers as principal investigators were mostly supported by relatively large, project-type research support systems such as "21st century COE (Ministry of Education, Culture, Sports, Science and Technology)," "Grant-in-Aid for Scientific Research on the Priority Area (Ministry of Education, Culture, Sports, Science and Technology)," "Grant-in-Aid for Scientific Research (S) (Ministry of Education, Culture, Sports, Science and Technology)," "Research for the Future Program (JSPS)," "Exploratory Research for Advanced Technology Program (JST)" and "Rice Genome Research Program (Ministry of Agriculture, Forestry and Fisheries)."

6-2 Measures for further promoting the gene silencing

Measures for further promoting the gene silencing are discussed based on the characteristics of the area.

(1) Importance of basic research taking a problem-solving approach in a spiral-type research area

In gene-silencing research, breakthroughs that were derived from basic research have established the direction of the applied research toward medical application. To date, the advance in gene-silencing research has greatly contributed to the elucidation of life, but many functions and mechanisms remain unknown, especially the common mechanisms shared by all organisms. These include the regulation of gene expression in the development and differentiation processes.

Furthermore, the medical application of this technology requires the resolution of several research problems, which involves not only applied or clinical studies but also basic studies including the elucidation of gene networks.

The area of gene-silencing research does not fit a linear advance model of "basic research → applied research → clinical research" but fits a spiral advance model of "basic research → applied research → clinical research → basic research → applied research → clinical research → " or a braid-type model in which the area is advanced through intertwining of "basic research", "applied research" and "clinical research".

The area is currently approaching the end of the first spiral. It is essential to start basic research taking the problem identification and problem-solving approach forward to the second spiral.

(2) Establishment of an environment promoting spontaneous adoption of research from other areas

As can be seen from the fact that the gene-silencing research started from the area of plant research, breakthroughs that expanded the research area were derived from the adoption of research from other areas. Therefore, it is important to establish an environment promoting information exchange between various research areas.

For example, a funding agency decides a subject for research adopting a task-solving approach and recruits about 10 research groups taking different approaches and belonging to different research areas. About twice a year, the agency hosts interim meetings where the research groups can exchange their results. The agency does not enforce joint research among the groups but only promotes information exchange.

Alternatively, it may be effective to organize a research meeting for researchers gathered from various areas. As in the Gordon Research Conference, the participants selected by the committee congregate together and thoroughly debate a particular research issue in a closed environment.

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